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## Neuronal differentiation of embryonic stem cells.

van Inzen WG, Peppelenbosch MP, van den Brand MW, Tertoolen LG, de Laat SW.

Hubrecht Laboratory, Netherlands Institute for Developmental Biology, Utrecht, The Netherlands.

Neuronal differentiation from totipotent precursors in vitro, is thought to require two signals: first a biophysical state (cellular aggregation) followed by a biochemical signal (retinoic acid treatment). In investigating the properties of retinoic acid-differentiated embryonic stem cell lines. However, we noted that retinoic acid treatment without prior aggregation, is sufficient to induce expression of the neuronal markers GAP-43 and NF-165. In agreement, immunohistochemistry revealed the presence of GAP-43 positive cells in these embryonic stem cell monolayers after three days of retinoic acid (RA) treatment. Furthermore an NF-165 positive subpopulation of cells was clearly observed after 4-5 days of RA treatment. The expression of these neuronal markers coincided with the appearance of electrically excitable cells, as assayed with whole cell patch clamp recording. We conclude that for neuronal differentiation of totipotent embryonic stem cells in vitro, one biochemical signal, i.e. retinoic acid treatment, is sufficient.

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